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Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L)

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Abstract: The fumigant toxicity of various volatile constituents of essential oils extracted from sixteen Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* L (Coleoptera: Curculionidae), was determined. The most potent toxicity was found in the essential oil from *Mentha arvensis* L var *piperascens* (LC_{50} = 45.5 μl litre^{-1} air). GC–MS analysis of essential oil from *M arvensis* showed it to be rich in menthol (63.2%), menthone (13.1%) and limonene (1.5%), followed in abundance by β-pinene (0.7%), α-pinene (0.6%) and linalool (0.2%). Treatment of *S oryzae* with each of these terpenes showed menthone to be most active (LC_{50} = 12.7 μl litre^{-1} air) followed by linalool (LC_{50} = 39.2 μl litre^{-1} air) and α-pinene (LC_{50} = 54.9 μl litre^{-1} air). Studies on inhibition of acetylcholinesterase activity of *S oryzae* showed menthone to have a nine-fold lower inhibitory effect than menthol, despite menthone being 8.1-fold more toxic than menthol to the rice weevil. Different modes of toxicity of these monoterpenes towards *S oryzae* are discussed.

Keywords: *Sitophilus oryzae*; rice weevil; fumigant toxicity; essential oils; *Mentha arvensis*; menthone; menthol; acetylcholinesterase inhibition

1 INTRODUCTION

Infestation of grain by various stored-product pests may occur at various stages from time of harvest to procurement by consumers. The susceptibility of stored grain to insect infestation is dependent on a number of factors, including condition of the grain at harvest, environment (ie temperature, humidity, etc), cleanliness of on-farm and bulk storage facilities, and treatment of the grain by protectants, usually fumigants. The grain industries in developing countries use a range of methods to minimize insect infestation on stored grains, but generally rely on fumigants.[1]

Protectants or insecticides are important tools in the integrated management of insect pests of stored grains. These chemicals are used extensively by all sectors of the grain industry. Currently, a mixture of organophosphate (OP) and pyrethroid insecticides, with piperonyl butoxide added as a synergist, is also used to control stored-grain insect pests. However, field populations of stored-grain insect pests have markedly increased in their resistance to OP insecticides over the past decade.[2–4] Thus, if current strategies of using these pesticides to control insect pests of grain continue, increasing amounts of pesticide will be required, possibly rendering grain pests completely resistant to the pesticides.

In many storage systems the use of fumigants is the most economical tool for managing stored-grain insect pests.[5] Ideal fumigants should leave no residues hazardous to humans and should not adversely affect the nutritional quality, flavour, or processing characteristics of stored grains.[1] Fumigants should be biologically active, sufficiently volatile to be removed by aeration, not absorbed by grain, not flammable and not corrosive. Currently, few chemicals are available for use as fumigants that meet these constraints. Methyl bromide, currently the most effective fumigant, will soon be restricted due to its potential ozone-depleting properties.[6] Moreover, it is highly toxic to warm-blooded animals.[7] The fumigation of stored grains with phosphine is likely to become more widely used in the future because of its efficacy and rapid desorption. However, phosphine fumigation may become increasingly limited in use because resistance of stored grain insects to phosphine has now been discovered in more than 45 countries.[8,9] In addition, phosphine has been argued to be genotoxic to occupationally exposed fumigators.[10] Because of the

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increasing drawbacks in continued use of today's conventional fumigants an effort is needed to develop new compounds to replace those currently used.

Essential oils are potential sources of alternative compounds to currently used fumigants. Essential oils have low toxicity to warm-blooded animals, high volatility and toxicity to stored-grain insect pests.[11–13] Essential oils are easily distilled from suitable plant material. Their major constituents, monoterpenes, are also of interest to industrial markets because of other potent biological activities in addition to their toxicity to insects.[14–19]

Herein, we report toxicities of essential oils as fumigants from sixteen Korean medicinal and spice plants, and of monoterpenes identified from the most active essential oil of *Mentha arvensis* L var *piperascens*, against the rice weevil, *Sitophilus oryzae* (L). Rice weevils are one of the chief stored-grain pests, worldwide.[20–22] We also report the inhibition by monoterpenes of acetylcholinesterase activity in *S oryzae* to establish a primary mode of action for their toxicity.

2 METHODS AND MATERIALS

2.1 Insects

Rice weevils were from a colony maintained by the Department of Agricultural Biology, Korea University, Korea. Weevils were reared to the adult stage using techniques described by Ryoo and Cho.[23]

2.2 Essential oils from Korean medicinal plants

Sixteen Korean medicinal plants were purchased from Kyeng-Dong traditional market in Seoul, Korea, and identified by Professor KH Yoon, Department of Biology, Soonchunhyang University, Asan, Korea. After drying at room temperature, leaves of the medicinal plants were subjected to steam distillation according to protocols outlined by Marcus and Lichtenstein.[24] Species and yields (%) of essential oils of each dried plant are: *Adenophora remotiflora* M (Campanulaceae), <0.01%; *Adenophora triphylla* var *japonica* Hara (Campanulaceae), <0.01%; *Artemisia princeps* var *orientalis* Hara (Asteraceae), 0.54%; *Aster scaber* T (Compositae), <0.01%; *Caranga sinica* R (Annonaceae), <0.01%; *Chrysanthemum coronarium* L var *spatiosum* Benth (Compositae), <0.01%; *Chrysanthemum zawdskii* var *latilobum* K (Compositae), 0.24%; *Crisium japonicum* var *ussuriense* K (Compositae), <0.01%; *Leonurus sibiricus* L (Lamiaceae), 0.04%; *Liriope muscari* Benth (Liliaceae), <0.01%; *Lonicera japonica* Thunb var *japonica* H (Caprifoliaceae), <0.01%; *Mentha arvensis* L var *piperascens* M (Labiatae), 1.05%; *Perilla frutescens* (L) Britt var *orientalis* (Labiatae), 0.21%; *Pimpinella bursa-pastoria* M (Araliaceae), <0.01%; *Pueraria thunberginana* Benth (Leguminosae), <0.01%; *Taraxacum platycarpum* Darlst (Asteraceae), 0.02%.

2.3 Chemicals

Monoterpenes used in bioassays were purchased from Aldrich Co (Milwaukee, WI). Acetylthiocholine iodide (ATChI) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Co (St Louis, MO). All chemicals were of highest grade commercially available.

2.4 General procedure

Identification of constituents in essential oils was determined using electron impact mass spectra (EI-MS) obtained by gas chromatography–mass spectrometry (GC–MS). The GC–MS identification of terpenes was confirmed by co-injection with authentic samples. GC–MS was performed on a Shimadzu CLASS 5000 system interfaced with a Shimazdu QP-5000 GC–MS fitted with a CBP-5 (25 m × 0.25 mm ID) capillary column. Chromatography conditions were as follows: column temperature, profile 70 °C to 80 °C at 1 °C min^{-1}, to 190 °C at 10 °C min^{-1}, and then to 250 °C at 30 °C min^{-1}; injector temperature, 250 °C; detector temperature, 280 °C; carrier gas, helium at 30 cm s^{-1}.

2.5 Fumigation bioassays

Fumigation chambers were 3.4-liter glass round-bottom flasks with glass stoppers fitted with a hook. Test fumigant materials deposited on a piece of filter paper (Whatman No 1) were placed, together with a small cage containing insects, into the fumigation chamber. The aqueous solution of terpenes were made freshly before bioassay. To obtain even distribution of test fumigants during assays, a magnetic stirrer was used at the bottom of each flask. Twenty adult insects, aged 10–15 days, were placed in each of three cages (4 cm in length and 1.5 cm ID) perforated with small holes to enable penetration of any volatiles emanating from the test source. Small amounts of polished rice were placed in each cage as a food source for the weevils. Each treatment was duplicated. Mortality was determined after 24 h of treatment. The LC_{50} and LC_{95} values were calculated by Probit analysis.[25] Control mortality was accounted by Abbott's formula.[26]

2.6 Inhibition study

Inhibition of acetylcholinesterase (AChE) was assessed by the method of Ellman *et al.*[27] Test monoterpenes were dissolved in absolute ethanol (5 ml) and the total volume made to 50 ml with 0.1 M phosphate buffer, pH 8.0, to give a 0.09 M stock solution of each test compound. Assay concentrations of monoterpenes were diluted serially so that the concentration of ethanol never exceeded 10 ml $litre^{-1}$, a concentration we assessed to inhibit AChE by less than 5%. Each assay was repeated three times. The reaction mixture contained a crude AChE enzyme preparation from adults of *S oryzae* (100 µl), ATChI (0.5 mM), DTNB (0.33 mM) and phosphate buffer (92.7 mM) containing the test monoterpene, to a total volume of 3 ml. The reaction was initiated by addition of crude enzyme, followed by incubation at 25 °C for 5 min. Levels of AChE activity were estimated by determining levels of

thiocholine through measuring absorbance at 412 nm. All test and control assays were corrected using blanks for non-enzymatic hydrolysis of ATChI.

3 RESULTS

3.1 Fumigant toxicity of essential oils

Toxicities of volatile compounds from essential oils of Korean medicinal and spice plants are shown in Table 1. Two essential oils, one from *M arvensis* (LC_{50} = 45.5 μl litre^{-1} air) and the other from *P bursa-pastoria* (LC_{50} = 89.0 μl litre^{-1} air), had potent fumigant activities against the rice weevil. The oils from *A princeps, C zawadskii, L sibiricus, P frutescens* and *T platycarpum* showed only weak activity (LC_{50} > 150 μl litre^{-1} air) against the insects.

3.2 Analysis of *Mentha arvensis* essential oil components and their fumigant activity

Analysis of essential oil from *M arvensis* showed it to be rich in menthol (**1**) (63.2%), menthone (**2**) (13.1%), limonene (**3**) (1.5%), followed by β-pinene (**4**) (0.7%), α-pinene (**5**) (0.6%) and linalool (**6**) (0.2%) (Fig 1). Menthone was the most biologically active fumigant (LC_{50} = 12.7 μl litre^{-1} air) found in *M arvensis* oil against the weevils, followed by linalool (LC_{50} = 39.2 μl litre^{-1} air) and α-pinene (LC_{50} = 54.9 μl litre^{-1} air) as shown in Table 1.

3.3 Inhibitory effects of monoterpenes on AChE activity

Of the monoterpenes examined, the most potent inhibitor of AChE from *S oryzae* was β-pinene (K_i = 0.0028 mM) followed by menthol (K_i = 0.048 mM) (Table 2). However, despite menthone having the highest toxicity towards the weevils, it showed a nine-fold lower inhibitory effect on AChE activity (K_i = 0.39 mM) than menthol. Dixon plots of inhibition on AChE activity by menthol and menthone are shown in Fig 2. These plots showed these monoterpenes to be competitive inhibitors of AChE in that increasing inhibition was associated with decreasing substrate concentration.

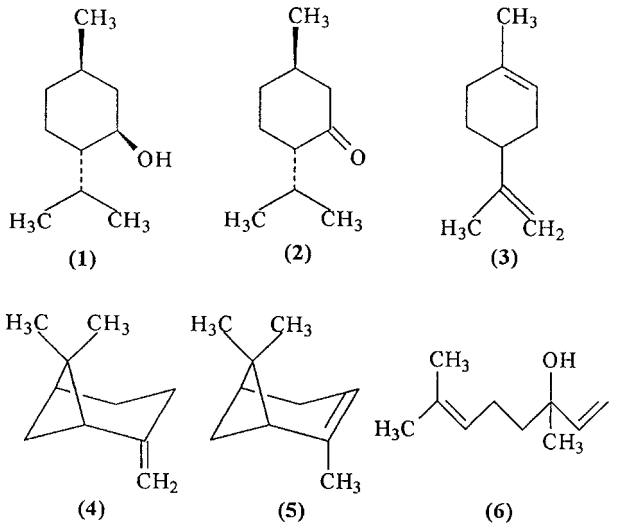

Figure 1. Structures of monoterpenes isolated from the essential oil of *Mentha arvensis*: (**1**) menthol, (**2**) menthone, (**3**) limonene, (**4**) α-pinene, (**5**) β-pinene, (**6**) linalool.

3.4 Fumigant toxicity of commercial monoterpenes and their inhibitory effects on AChE activity

In addition to activities of monoterpenes isolated from the essential oils, toxicities and AChE inhibition (K_i

Plant species or monoterpenes	LC_{50} (μl litre^{-1} air)	LC_{95} (μl litre^{-1} air)
Adenophora remotiflora	—[a]	—
Adenophora triphylla var *japonica*	—	—
Artemisia princeps var *orientalis*	>150	>150
Aster scaber	—	—
Caranga sinica	—	—
Chrysanthemum coronarium var *spatiosum*	>150	>150
Chrysanthemum zawdskii var *latilobum*	>150	>150
Crisium japonicum var *ussuriense*	—	—
Leonurus sibiricus	>150	>150
Liriope muscari	>150	>150
Lonicera japonica var *japonica*	>150	>150
Mentha arvensis var *piperascens*	45.5 (40.0–51.0)	125 (101–156)
Perilla frutescens var *orientalis*	>150	>150
Pimpinella bursa-pastoria	89.0 (74.4–101)	254 (232–278)
Pueraria thunberginana	>150	>150
Taraxacum platycarpum	>150	>150
Limonene	61.5 (49.0–76.5)	141 (88.6–225)
Linalool	39.2 (35.3–43.4)	77.5 (63.4–94.6)
Menthol	148 (128–195)	830 (530–1680)
Menthone	12.7 (11.4–14.1)	25.1 (20.6–30.7)
α-Pinene	54.9 (37.5–44.2)	76.0 (45.0–128)
β-Pinene	78.9 (72.1–85.7)	107 (91.2–124)

Table 1. Toxicities of essential oils and principal monoterpenes of *Mentha arvensis* oil against *Sitophilus oryzae*

[a] —: not detectable.

Table 2. K_i values of monoterpenes identified in the essential oil of *Mentha arvensis* against acetylcholinesterase activity from *Sitophilus oryzae*

Monoterpenes	K_i *(mM)*
Limonene	0.73
Linalool	ND[a]
Menthol	0.048
Menthone	0.39
α-Pinene	0.44
β-Pinene	0.0028

[a] ND: not detectable.

values) of twelve additional monoterpenes against *S oryzae* are listed in Table 3. Of all monoterpenes tested, other than menthone (Table 1), isosafrole was the most toxic (LC_{50} = 19.3 µl litre^{-1} air) to the weevils, followed by cineole (LC_{50} = 23.5 µl litre^{-1} air), *p*-cymene (LC_{50} = 25.0 µl litre^{-1} air) and terpinen-4-ol (LC_{50} = 25.6 µl litre^{-1} air). Inhibition studies showed carvacrol (K_i = 0.050 mM) and methyl eugenol (K_i = 0.051 mM) to be equally inhibitory to AChE activity.

4 DISCUSSION

A large number of essential oils extracted from various spice and herb plants have already been screened for toxicity as potential fumigants. The oil under the code name ZP51 was found to be the most potent fumigant of all oils tested against four major stored-product insect pests.[11] ZP51 showed strong activity against *S oryzae* and *Tribolium castaneum* (Herbst) which were tolerant to all the other oils tested. At a concentration of 4.5 µl litre^{-1} air or less ZP51 caused 90% mortality against all test insects within 24 h. At a concentration of 30 µl litre^{-1} air and after a 2-day exposure, ZP51 caused 100% mortality of *S oryzae*. In comparison, our results indicate *M arvensis* essential oil to have promising fumigant toxicity against this insect. At a concentration of 45.5 µl litre^{-1} air and a 1-day exposure, the oil caused 50% mortality of *S oryzae*. Mortality may increase after longer exposure periods. Previously, two groups[28,29] have reported that the LC_{50} of *M arvensis* oil against 3-day-old adult weevils of *S oryzae* was 229.8, 136.8 and 118.8 µl litre^{-1} of air after 24, 48 and 72 h exposure, respectively. These results showed that longer exposure of insects to essential oil increased mortality. However, mortality rates determined by Srivastava *et al*[28] were significantly lower (fivefold) than those found in our study. This difference may be a result of different strains of *S oryzae* or differences in primary constituents in *M arvensis* essential oils used between the studies. Recently, Lee *et al*[30] reported that a chlorpyrifos-methyl-resistant strain of *Oryzaephilus surinamensis* L, another beetle species that is a stored-product pest, collected in Australia was cross-resistant to essential oil obtained from *Eucalyptus globulus* Labill and its primary monoterpene, 1,8-cineole. This resistant strain showed 1.9-fold and 2.2-fold higher tolerance against *E globulus* essential oil and 1,8-cineole, respectively, relative to a susceptible strain, VOS48, of *O surinamensis*. The elevated resistance was possibly related to higher levels of detoxifying enzymes, such as cytochrome P450-dependent monooxygenases and esterases in the resistant strain.

Joia and Kumar[31] found that susceptibility of *S oryzae* to malathion varied among populations collected from 33 different locations in Punjab, India. The levels of resistance in these geographic strains ranged from seven- to 18-fold greater in relation to a control, susceptible strain of *S oryzae*. Our study used

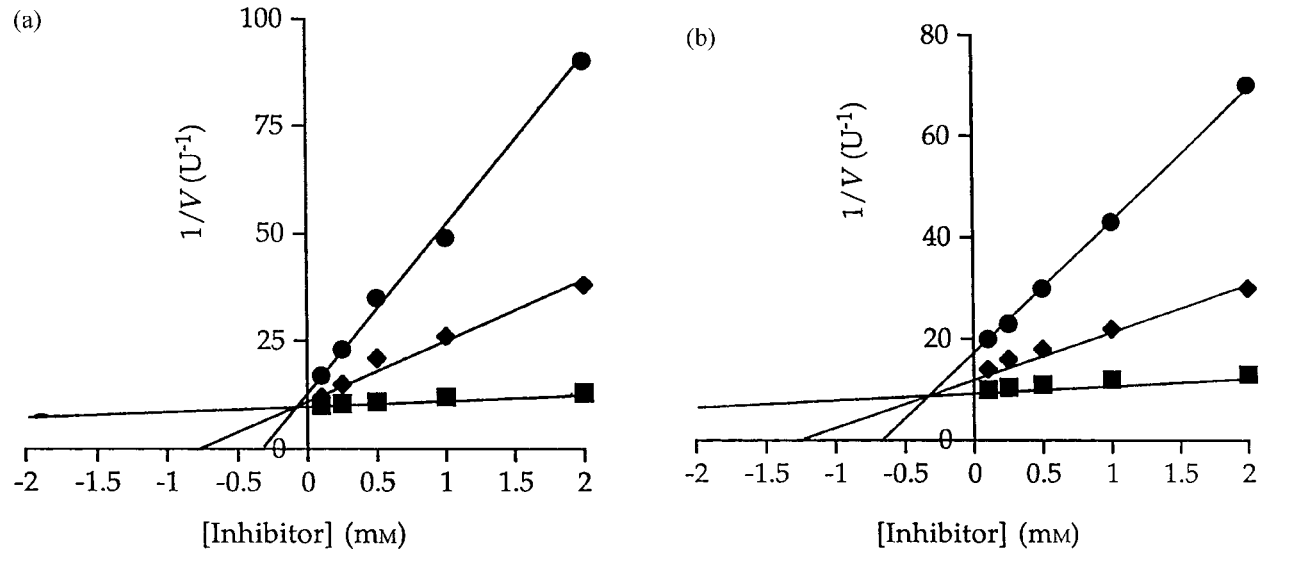

Figure 2. Dixon plots derived from inhibition of acetylcholinesterase from *Sitophilus oryzae* by (a) menthol and (b) menthone. In each plot the concentrations of ATChI are (●) 0.055 mM, (◆) 0.167 mM and (■) 0.50 mM.

Monoterpenes	LC_{50} (μl litre^{-1} air)	LC_{95} (μl litre^{-1} air)	K_i (mM)
Carvacrol	79.7 (67.6–93.6)	402 (297–625)	0.050
Cineole	23.5 (17.5–31.9)	44.2 (31.5–62.0)	0.084
p-Cymene	25.0 (23.3–26.5)	39.0 (35.7–44.3)	2.23
Eugenol	50.7 (45.3–57.5)	129 (103–184)	0.096
Isoeugenol	>150	>150	0.11
Isosafrole	19.3 (14.0–26.7)	78.3 (51.5–119)	0.71
Methyleugenol	70.8 (51.0–101)	259 (91.0–738)	0.051
Perilla aldehyde	38.4 (32.0–44.2)	131 (104–191)	1.33
α-Terpinene	71.2 (64.2–85.7)	179 (143–258)	0.14
α-Terpineol	69.1 (54.7–87.4)	165 (86–224)	3.94
Terpinen-4-ol	25.6 (21.0–31.0)	66.4 (46.1–95.3)	ND[a]
Thymol	69.7 (62.3–78.9)	174 (137–260)	0.57

[a] ND: not detectable.

Table 3. Toxicity and acetylcholinesterase inhibitory activity (K_i) of selected monoterpenes against *Sitophilus oryzae*

a strain of *S oryzae* different from the Indian field strain used in the study of *M arvensis* essential oil toxicity by Srivastava *et al.*[28] We suggest that the difference in susceptibility of *S oryzae* in our study compared with that of Srivastava *et al*[28] was possibly that the latter study used an insecticide-resistant strain cross-resistant to essential oils of *M arvensis*. The strain of *S oryzae* used in our study is highly susceptible to insecticides (Prof KJ Cho Pers comm). An additional possibility to explain the difference of the *S oryzae* response to essential oils from that of *M arvensis* in our study and that of Srivastava *et al*[28] is a difference in the constituents of the essential oils. However, Srivastava *et al*[28] did not analyze the composition of the oil. Differences in environmental effects during the cultivation of *M arvensis* could have led to differences between the constituents of the oils used in the two studies. However, our results showed the primary monoterpenes of essential oil obtained from *M arvensis* were similar in proportion to a previous report.[32]

The toxicity of a number of monoterpenes has been evaluated against various stored-product insects. Coats *et al*[33] found that exposure of *S oryzae* for 24 h to linalool and *d*-limonene gave LC_{50} values of 14 and 19 μl litre^{-1} air, whereas the LC_{50} values for myrcene and α-terpineol were >100 μl litre^{-1}. Our results showed different toxicities for linalool (LC_{50} = 39.2 μl litre^{-1} air), limonene (LC_{50} = 61.5 μl litre^{-1} air) and α-terpineol (LC_{50} = 69.1 μl litre^{-1} air) against the rice weevil from those found in that study.[33] As we mentioned previously, this difference may result from the use of different strains of rice weevil.

A relationship between monoterpene toxicity, inhibition of AChE activity and insecticidal effects was previously reported.[34–36] However, our results did not show that terpenoid toxicity is necessarily correlated with ability to inhibit AChE activity. The most toxic monoterpene in our study, menthone, was only weakly inhibitory of AChE activity compared with menthol which was only weakly toxic but showed 8.1-fold greater inhibitory activity against AChE than menthone. This finding suggests that there may be different modes of action of monoterpene toxicity to *S oryzae*. One possibility for the differences in toxicity between menthol and menthone could be differences in liphophilicity and volatility. One other possibility is that some monoterpenes may inhibit cytochrome P450-dependent monooxygenases.[37]

Mentha essential oil shows potent toxicity to the rice weevil. The primary component of *Mentha* oil, menthone, was found to be the principal toxic constituent. Hence, menthone may show some promise as an alternative to those fumigants currently used to control storage grain insect pests. Moreover, menthone has been found to have antiallergic,[16] antimicrobial[14] and spasmolytic properties,[15] suggesting this natural monoterpene could be a safer fumigant to control stored-grain insect pests than those currently used.

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